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Research Reports

Anatomical and behavioral analyses of the inheritance of audiogenic seizures in the progeny of genetically epilepsy-prone and Sprague–Dawley rats

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Our previous studies have shown an increase in the number of GABAergic and total neurons in the inferior colliculus (IC) of the genetically epilepsy-prone rat (GEPR-9) as compared to the non-seizing Sprague–Dawley (SD) rat. To determine whether an increase in neuron number in the IC is genetically associated with seizure behavior, seizing and non-seizing offspring of GEPR-9 and SD progenitor strains were studied as well as offspring from backcrosses made with F_1 and either GEPR-9 or SD rats. In addition, the ontogeny of seizure behavior was studied in seizing rats from these same backgrounds. The development of seizure behavior in GEPR-9s was shown to be dependent on age and the number of exposures to sound stimulus up until the age of 9 weeks. The F_1 and F_2 generations displayed different audiogenic seizure profiles than those of the two progenitor strains. In the F_1 generation, the ratio of seizing to non-seizing rats was always greater than 3:1, and the distribution of seizure scores was similar for males and females. In addition, the offspring from backcrosses made with F_1 rats (high or low seizing) and GEPR-9s displayed maximal audiogenic response scores (ARS) of 9, a characteristic of the GEPR-9s used in this study. The results of these genetic studies indicate a polygenic inheritance of this autosomal dominant trait of audiogenic seizure susceptibility.

For the quantitative study of neuronal density in the IC, neurons were counted from cresyl violet-stained preparations from seizing and non-seizing F_1 and F_2 rats, backcrosses from different categories and age-matched SD rats. Statistically significant increases in the number of both small (70% increase) and medium-sized (14% increase) neurons occurred in the high seizing animals (ARS = 7–9) as compared to either the non-seizing F_2 or SD rats. In addition, a significant increase in the number of small neurons (77% increase) occurred in the high seizing offspring of the $F_1 \times$ GEPR-9 backcross as compared to that of the non-seizing offspring of the $F_1 \times$ SD backcross. The data from 25 rats generated a 0.9 coefficient of linear correlation between ARS and the number of small neurons. The results from the anatomical studies suggest that the inheritance of audiogenic seizures appears to be closely linked to the increase in cell number. Therefore, the increase in cell number in the IC may be an important determinant of seizure behavior for GEPR-9s.

INTRODUCTION

The genetically epilepsy-prone rat (GEPR-9) exhibits severe generalized motor seizures in response to intense auditory stimuli^{1,4}. Results of le-

sion studies indicate that the primary neuronal pathways involved in the manifestation of audiogenic seizures are subcortical because partial or total ablation of cortex does not prevent seizures³ whereas bilateral lesions of the inferior colliculus (IC) and brain-stem reticular formation prevent seizures^{2,15,16,31}. Other data show that the IC is abnormal in the GEPR-9. For example, results of pharmacological studies indicate that neurons in

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the IC of the GEPR-9 may be less sensitive to GABA and benzodiazepine iontophoresis than neurons in the IC of control rats^{5,6}. Also, an increase in afterdischarge-like responses similar to that observed in other types of seizures has been observed in the IC of the GEPR-9⁷. We have previously shown that the IC of adult GEPR-9s displays an increase in the total number of neurons and an increase in the number of GABAergic neurons as well²⁵. In addition, we have shown a significant increase in the levels of GABA in the central nucleus of the IC of GEPR-9s as compared to SD rats in a recent biochemical study²¹. The increase in cell number within the IC is present prior to seizure activity because an increase in the total number of neurons has been demonstrated in GEPR-9s at an age prior to the onset of seizures²⁴. Therefore, the increase in the total number of neurons and in the number of GABAergic neurons is not caused by seizure activity or exposure to an acoustic stimulus. A similar increase in the number of GABAergic neurons was observed in the hippocampal dentate gyrus of another model of genetic epilepsy¹⁹, and interruption of the entorhinal afferents to this structure will block seizures²². Thus, increased numbers of GABAergic neurons could be a determinant of seizure behavior in some genetic models of epilepsy.

In the present study we wanted to determine whether the increase in cell number is genetically associated with audiogenic seizure behavior. Thus, we have crossed Sprague–Dawley (SD) rats, that are non-susceptible to seizures following acoustic stimuli, with rats from our GEPR-9 (maximal seizures) colony to produce an F₁ generation. An F₂ generation was produced by mating various F₁ animals that had different seizure susceptibilities. In addition, backcrosses were produced. The number of neurons in the IC of these different offspring were counted and correlated with the level of seizure susceptibility. Since only a brief description of the development of seizure behavior has been reported in GEPR-9s^{1,20} and no other data have been reported on the behavior of the progeny of GEPR-9s and SD rats, this study will also describe the development of seizures in GEPR-9s, F₁, F₂ and backcross progeny.

METHODS

All animals received a series of exposures to intense auditory stimuli (2 electric doorbells) and the seizure behavior was rated according to the scale established by Jobe et al.¹⁴. Briefly, the audiogenic response scores (ARS) range from 0 to 9, with 0 equal to no seizure and 9 equal to a maximal, tonic-clonic seizure. Rats that scored 7–9 on this scale were referred to as *high-seizing*, whereas others that scored 1–3 were classified as *low-seizing*. The GEPR-9s used in this study were from the same colony as those used in our previous studies^{21,24,25}. GEPR-9s and Sprague–Dawley (SD) rats were bred to produce F₁ progeny. F₁ animals were tested and those which exhibited wild running (ARS = 1) or no seizures were bred to produce several litters of F₂ progeny. Backcrosses were produced by mating high-seizing F₁ rats with GEPR-9s, low-seizing F₁ rats with GEPR-9s, high-seizing F₁ rats with SD rats or low-seizing F₁ rats with SD rats. All GEPR-9s were given at least 3 tests that were administered at 3 week intervals beginning at: (1) 18 days of age, (2) 3 weeks, (3) 6 weeks, or (4) 9 weeks. Members of the F₁ and F₂ generations were tested at least 6 times at similar ages to the GEPR-9s. Members of the various backcrosses were also tested at least 4 times at similar ages to the GEPR-9s. SD rats received 3 tests at 3, 6 and 9 weeks of age and they were shown to be non-susceptible to seizures following acoustical stimuli.

After seizure records were obtained for the progeny of the different crosses and backcrosses, the animals at an age of 100–120 days were deeply anesthetized with Nembutal and intracardially perfused with 0.9% saline followed by a 4% paraformaldehyde solution in phosphate buffer (pH 7.4). The brains were dehydrated in alcohol, embedded in paraffin and sectioned in the coronal plane at a thickness of 10 μ m. Every tenth section throughout the midbrain was stained with cresyl violet and analyzed. Samples from both right and left colliculi from 10 to 12 levels throughout the rostrocaudal extent of the IC were examined.

The somata of neurons were counted from a representative 62,500 μ m² grid in the ventral lateral

portion of the central nucleus of the IC from 29 rats. The quantitative analysis included 5 high-seizing (ARS = 7) and 4 non-seizing rats (ARS = 0–1) from the F_2 generation, 4 high-seizing backcrosses (ARS = 9) from high-seizing $F_1 \times$ GEPR-9, 4 high-seizing backcrosses (ARS = 9) from low-seizing $F_1 \times$ GEPR-9, 3 high-seizing backcrosses (ARS = 7–9) from high-seizing $F_1 \times$ SD, 5 low-seizing backcrosses (ARS = 0–2) from low-seizing $F_1 \times$ SD, and 4 age-matched SD rats. The exact location of this analysis and criteria for neuronal identification have been described previously^{23–25}. Somata were classified as small (<15 μ m in diameter), medium (15–25 μ m in diameter) or large (>25 μ m in diameter) and the average cell number in each size group was tabulated. The counting of neurons was conducted by the same individual in a single blind study.

Data were statistically analyzed using Student's *t* test when only two groups were compared. To determine whether significant differences existed between the various data groups, a 1-way analysis of variance for these groups was initially used. When the sample populations were small, a non-parametric test, the Kruskal–Wallis method, was employed. If significant differences were found between the groups, then an unpaired *t* test or a Mann–Whitney U test was used to determine the significance of the differences between two of the groups. If differences were observed, they were expressed relative to the non-seizing SD rats.

RESULTS

Seizure phenotypes of offspring from crosses

The GEPR-9 \times SD rat reciprocal crosses yielded a consistently large number of seizing offspring (ARS = 1–9) with only a few non-seizing (ARS = 0) offspring. The ratio of seizing to non-seizing offspring from the 4 litters generated for this study was at least 3:1. Both GEPR-9 \times SD and SD \times GEPR-9 reciprocal crosses were made. The ARS range was divided into 3 categories (high, medium and low or non-seizing). All F_1 offspring were placed into one of these categories and their sexes were determined (see Table I). The data showed that the distribution of ARS among male and female offspring was similar. The use of low-seizing

TABLE I

F₁ seizure profiles of 3 litters obtained from reciprocal crosses

Litters B and C were progeny from a GEPR male \times SD female cross whereas litter A was derived from the reciprocal cross SD male \times GEPR female. Note that the distribution of audiogenic response scores obtained at 11 weeks of age does not show a greater proportion of males with highest seizure scores. These results indicate that the genes for audiogenic seizures are autosomal.

Sex	ARS		
	Low-seizing (0–1)	Mild-seizing (2–4)	High-seizing (5–9)
A Male (n = 3)	2	0	1
Female (n = 2)	0	1	1
B Male (n = 7)	0	5	2
Female (n = 5)	0	3	2
C Male (n = 6)	3	3	0
Female (n = 5)	1	0	4

F_1 rats from these litters yielded F_2 litters that had a wide range of seizure scores.

The ARS of the offspring from the backcrosses were in the range that was expected from the apparent autosomal dominance of this seizure phenotype. Thus, crosses between high- and low-seizing F_1 rats and GEPR-9s yielded all high-seizing offspring (ARS = 9). The cross between high-seizing F_1 rats and SD rats also yielded high-seizing progeny (ARS = 7–9). In contrast, the backcross between low-seizing F_1 rats and SD rats yielded low- and non-seizing rats (ARS = 0–2).

Behavioral tests

The percentage of GEPR-9s receiving an ARS of 9 increased with age and previous experience until approximately 11 weeks of age. This percentage was determined at different ages ranging from 18 days to 9 weeks. At 18 days of age no animals had seizures, whereas at 9 weeks of age 100% of the rats exhibited maximal seizures (ARS = 9), regardless of the number of previous tests (Figs. 1a and b). At intermediate ages, however, the number of exposures to sound stimulation, as well as the age of the animal, influenced the animal's seizure score. For example, at 3 weeks of age, 21% of

the animals that received their first test had an ARS of 9, whereas 33% of the rats that received their second test had an ARS of 9 (Fig. 1a).

An interesting phenomenon was observed in the

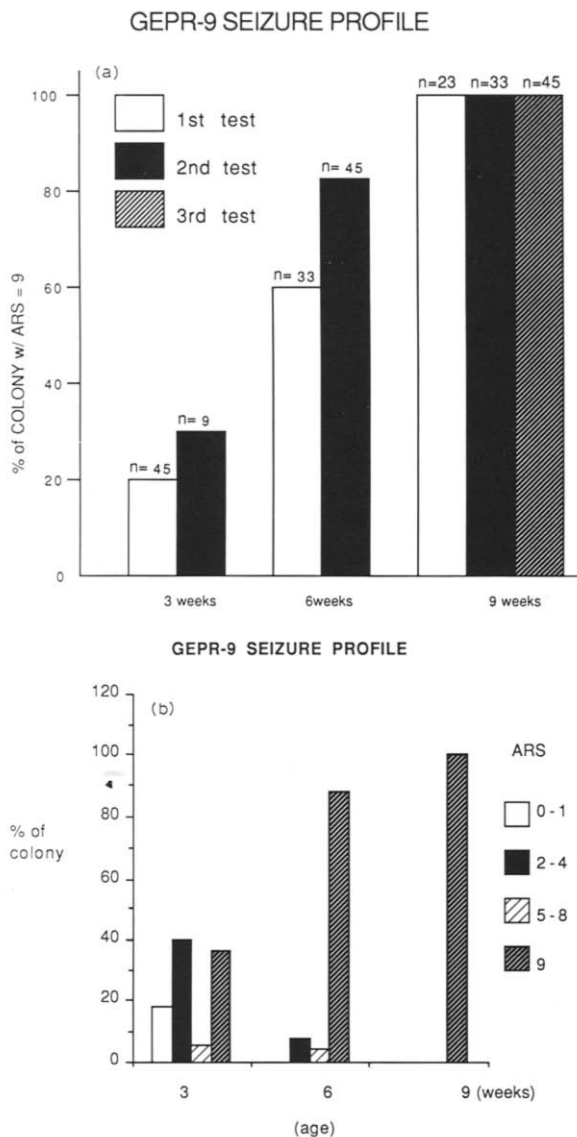


Fig. 1. a: histogram showing the percentage of offspring from GEPR-9 parents scoring an ARS of 9 after sound stimulation at various ages. Note that both age and seizure experience influence the percentage of rats exhibiting a maximal seizure (ARS = 9). Also, by 9 weeks of age 100% of these rats have an ARS of 9 regardless of the number of previous tests. The numbers above each bar indicate the number of animals tested. b: histogram showing the percentage of offspring from GEPR-9 parents exhibiting various grades of seizure activity at specific ages. By 9 weeks of age 100% of these rats have an ARS of 9. These data can be compared with data in Fig. 2 for F₁ offspring.

GEPR-9s that were tested at 3 weeks of age. Approximately, 27.2% of the rats exhibited a postictal aggression response that usually lasted for 15–25 min. Behavioral changes included hyperactivity, persistent loud vocalization, and acts of aggression and violence against human hands, other cage mates and inanimate objects, such as the cage and water bottle. Although this response tended to occur more often in rats that displayed seizures of low intensity (ARS = 2–3), the response was also observed in those with maximal seizure intensity (ARS = 9). This rage response disappeared by 6 weeks of age. A small percentage of rats died subsequent to this behavioral display; the survival rate was improved by leaving the rat in a padded chamber until the episode ended.

At 6 weeks of age, 60% of the rats that experienced their first test had an ARS of 9, whereas 80% of the rats that experienced their second test had an ARS of 9. As stated above, the seizure scores obtained on or after 9 weeks of age were consistently stable at an ARS of 9. All GEPR-9s exhibited an ARS of 9 and control SD rats did not have seizures. The results for the development of

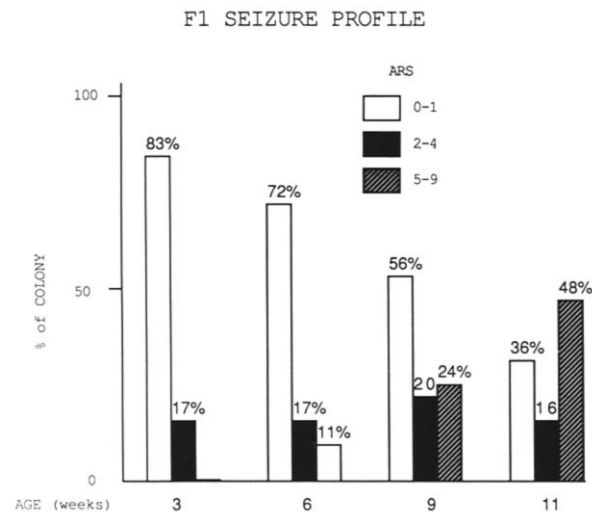


Fig. 2. Histogram showing the percentage of F₁ progeny exhibiting no seizure activity (ARS = 0–1), moderate seizures (ARS = 2–4) or severe seizures (ARS = 5–9) at various ages. The F₁ generation has audiogenic response phenotypes different from the progenitor strains. In addition, seizure intensity continues to vary after 9 weeks of age, whereas the ARS are stable in the GEPR after this time point. The number of animals (n) tested at each age was: 3 weeks of age, n = 12; 6 weeks of age, n = 18; 9 and 11 weeks of age, n = 25.

ARS in GEPR-9s are summarized in Fig. 1.

The seizure profiles of the F_1 and F_2 generations were very different from that of either the GEPR-9 or the SD progenitor strains. The F_1 and F_2 generations exhibited new audiogenic seizure response phenotypes rather than exhibiting the behavior of either of the parental strains (see Fig. 2). In addition, the most severe seizure behavior (ARS = 5–9) developed at a later age in the F_1 and F_2 generations than in the GEPR-9. Also, the seizure scores were not as consistent in these two generations (individual scores as well as between litter scores) as in the GEPR-9 colony. For example, at 3 weeks of age, 58% of the F_1 animals did not have seizures (ARS = 0–1), 42% of the rats exhibited moderate seizures (ARS = 2–4), and no animals had high seizures. At 6 weeks of age the seizure records were similar. In contrast to the GEPR-9 colony, there was no effect of prior experience on the seizure records in these animals at 6 weeks of age. High seizures first occurred at 8 weeks of age and at 9 weeks of age 36% of the rats had high seizures (ARS = 5–9). At this age, the percentage of rats that had low-seizure scores

dropped to 24%, and 40% of the animals did not display seizures. In contrast to the GEPR-9s, seizure behavior in F_1 rats did not remain stable after this time point. At 11 weeks of age the percentage of rats that had high seizures rose to 72% whereas the percentage of rats that had no or low seizures was 16% and 11%, respectively. Although the majority of F_1 rats had high seizure scores, only 10% ever received an ARS of 9, and none of them exhibited consistent scores of 9. In fact, the seizure scores of the animals seldom were consistent. The rats would often exhibit different scores on each test within the range of scores in either the high seizure category or the low seizure category.

The F_2 generations were produced from low-seizing (ARS = 1–2) and non-seizing (ARS = 0) F_1 parents with the aim of producing more individuals with an ARS of 0–1. The seizure profile of the F_2 rats was similar to that of the F_1 rats in that the F_2 progeny had different audiogenic phenotypes than either of the progenitor strains. Crossing non-seizing F_1 animals with each other produced a colony of animals in which a higher percentage of rats lacked seizures (41% of the F_2 vs. 16% of the F_1 rats). After several tests, approximately half the animals had seizures of high intensity in contrast to 72% of the F_1 rats and 100% of the GEPR-9s. At 3 weeks of age, 56% of the animals had never displayed seizures, whereas 13% had low-intensity seizures and 31% had high-intensity seizures. At 8 weeks, 56% of the animals had never exhibited seizures, 6% had low seizure scores and 38% had high seizure scores. At 10 weeks, 41% had never displayed seizures, 6% had low intensity seizures and 53% had high seizure scores. Similar to the F_1 strains, most high-seizing F_2 animals had an ARS of 7–8 and only 10% of the rats had an ARS of 9. In addition, there was much variability in the seizure scores between litters as well as in an individual's seizure profile. A summary of these results is found in Fig. 3.

It is important to indicate that the backcrosses were produced by each of the 4 possible ways, and they were treated as 4 separate groups (see Fig. 4). The seizure profiles of these backcrosses were very interesting. For example, 18% of the progeny from the F_1 high-seizing \times GEPR-9 cross showed maximal seizures (ARS = 9) at 3 weeks of age. At

F2 SEIZURE PROFILE

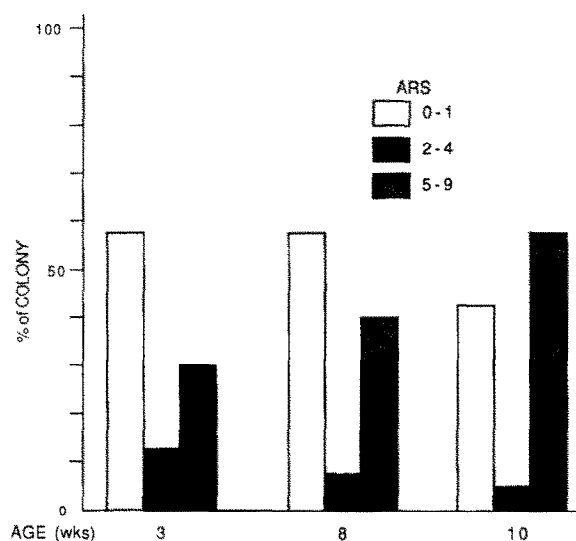


Fig. 3. Histogram showing the seizure profiles in several F_2 progeny produced from low-seizing F_1 animals. The F_2 strains have audiogenic response phenotypes that are different from the progenitor strains. The number of rats tested (n) at each age was 3 weeks of age, $n = 12$; 8 weeks of age, $n = 20$; 10 weeks of age, $n = 32$.

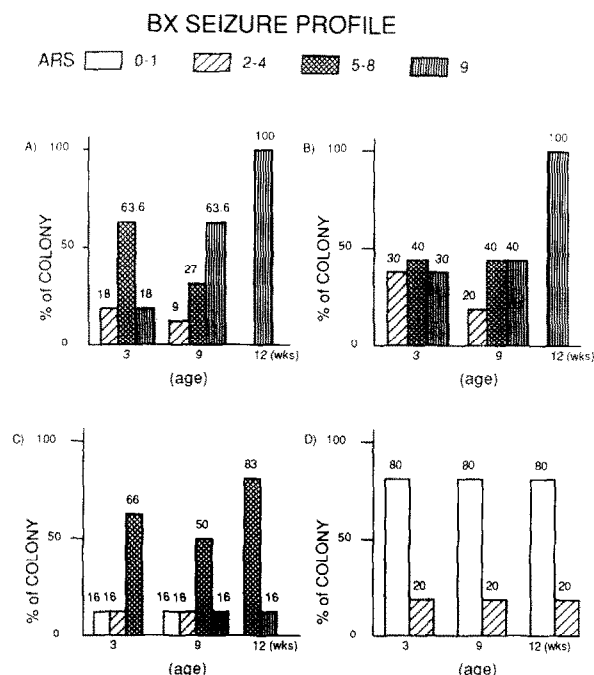


Fig. 4. Histogram showing the seizure profiles of progeny derived from the 4 backcrosses (BX). BX progeny have different audiogenic response phenotypes as compared to their progenitor strains. The numbers above each bar indicate the percentage of colonies. A, GEPR × F₁ (high), *n* = 11; B, GEPR × F₁ (low), *n* = 10; C, SD × F₁ (high), *n* = 6; D, SD × F₁ (low), *n* = 5.

9 weeks of age, 63% of this backcross displayed maximal seizures and at 12 weeks of age, all showed maximal seizures. All of the progeny of the second type of backcross (F₁ low-seizing × GEPR-9) exhibited maximal seizures at 11 weeks of age although at 3 weeks of age only 30% displayed this seizure type. In contrast, only 83% of the progeny from the third backcross (F₁ high-seizing × SD rat) displayed seizures with high intensity (ARS = 7–9) at 12 weeks of age. At 3 weeks of age, 33% of the offspring from this backcross displayed some seizure activity (ARS = 2). Finally, the last backcross (F₁ low-seizing × SD rat) displayed relatively no or low ARS throughout the entire testing period.

Anatomy

The Nissl preparations revealed a heterogeneous population of small, medium and large neurons in the central nucleus of the inferior colliculus (ICCN) of all animals (Fig. 5A–C). Statistically significant increases in the number of both small (70% increase) and medium-sized (14% increase)

neurons occurred in the high-seizing F₂ animals as compared to either the non-seizing F₂ or SD rats. Also, significant increases (77%) in the number of small neurons occurred in the high-seizing backcrosses compared to the backcross progeny that did not display seizures. Data are reported as mean ± standard error of the mean (Fig. 6). In the SD rats there was an average of 18.0 ± 0.72 small, 8.8 ± 0.54 medium and 1.9 ± 0.18 large neurons per unit area. The non-seizing F₂ had similar numbers of neurons in that there was an average of 20.4 ± 0.54 small, 8.4 ± 0.45 medium and 1.8 ± 0.45 large neurons per unit area. In contrast, the number of small and medium-sized neurons was greater in the high-seizing F₂ rats as compared to these latter two groups of rats. Thus, the high-seizing F₂ rats displayed 30.6 ± 1.0 small, 10.0 ± 0.52 medium and 1.4 ± 0.13 large neurons per unit area.

The data for the high-seizing progeny of the 4 backcrosses (Fig. 7) were consistent with the findings of the F₂ rats. Also, the high-seizing backcross progeny displayed significantly larger numbers of total neurons in the IC than the low-seizing backcross rats. A 1-way ANOVA was significant between groups and *t* test data indicated significant differences for each pair of groups for *small cell* number except for the 2 backcrosses that involved a GEPR-9 parent (see Fig. 7). Thus, the progeny from the first backcross (F₁ high-seizing × GEPR-9) displayed 41.7 ± 3.7 small, 10.8 ± 1.76 medium and 1.55 ± 0.32 large neurons per unit area. The second backcross group (F₁ low-seizing × GEPR-9) had 39.9 ± 2.27 small, 10.3 ± 1.08 medium and 1.69 ± 0.32 large neurons per unit area. The third backcross group (F₁ high-seizing × SD rat) had somewhat fewer neurons: 31.8 ± 1.54 small, 12.9 ± 1.7 medium and 1.35 ± 0.1 large neurons per unit area. In contrast to these backcrosses, the number of small neurons from the remaining backcross (F₁ low-seizing × SD rat) was significantly smaller than the previous 3 backcrosses; 23.2 ± 2.21 small, 11.3 ± 2.54 medium and 1.69 ± 0.72 large neurons per unit area. These increases in neuron number for the high-seizing groups in the F₂ and backcross progeny were similar in magnitude to those previously described in adult GEPR-9s²⁵.

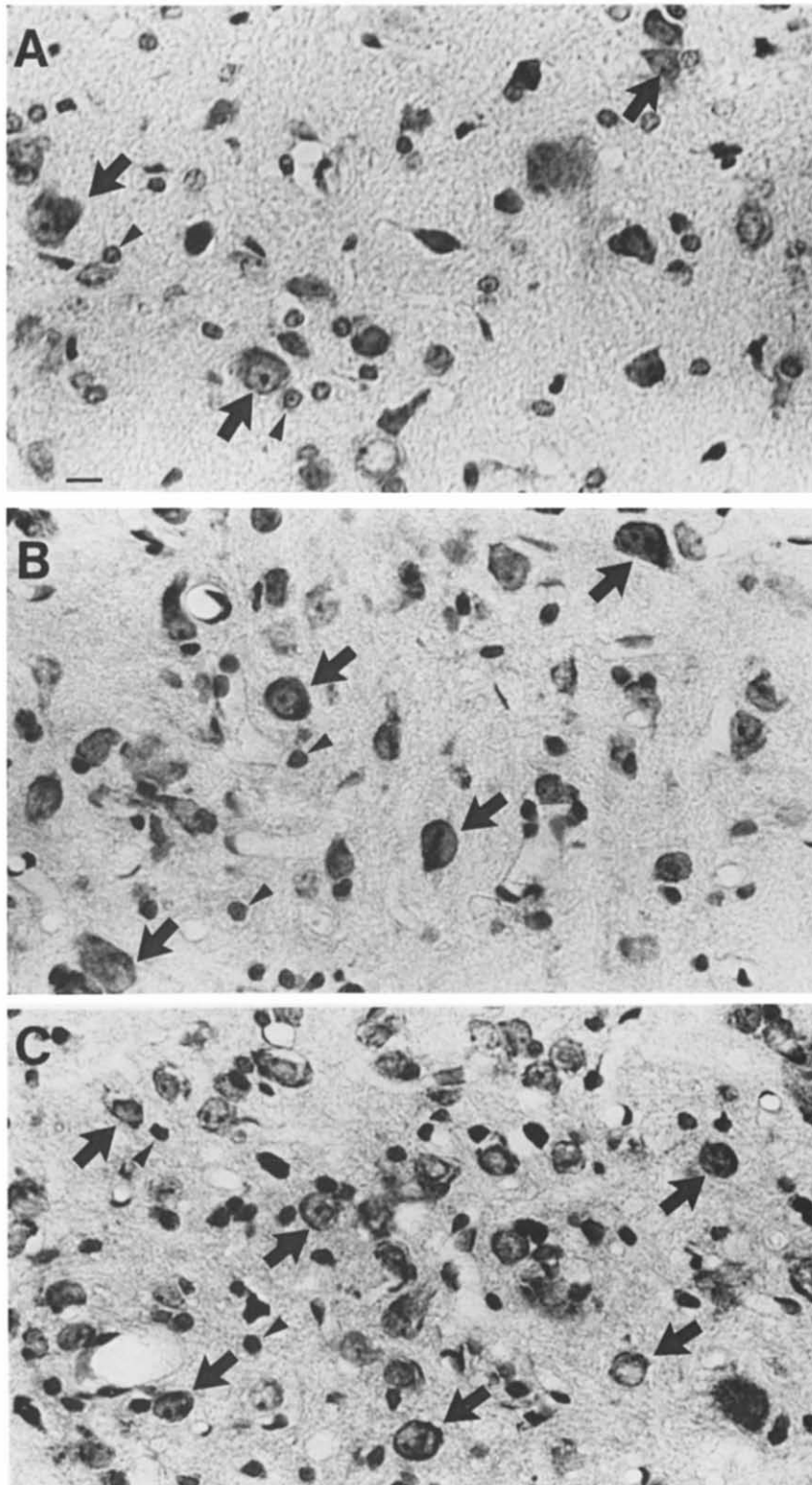


Fig. 5. Photomicrographs of Nissl preparations from representative regions from the ventral lateral portion of the central nucleus of the inferior colliculus of an SD rat (A), a non-seizing F_2 progeny (B) and a high-seizing F_2 progeny (C). Note that the number of neurons is similar for the SD rat and the non-seizing F_2 progeny. In contrast, the high-seizing F_2 progeny (C) displays an increase in the number of neurons. Arrows indicate neurons, arrowheads indicate glia. Scale bar = 10 μ m.

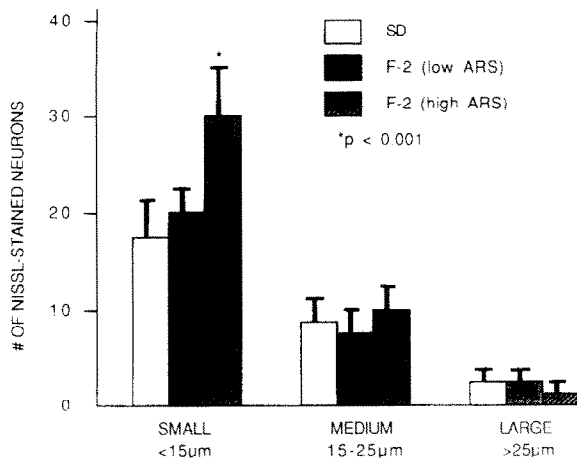


Fig. 6. Histogram showing the average number of small, medium-sized and large neurons counted from a representative area of the ventral lateral portion of the central nucleus of the inferior colliculus in seizing and non-seizing F_2 animals and age-matched Sprague-Dawley rats (SD). Error bars indicate standard deviations. The high-seizing F_2 rats showed a significant increase in the number of small neurons.

To determine whether a significant relationship existed between ARS and the number of small neurons in the IC, a coefficient of linear correlation was calculated (Fig. 8). A 0.9 value was obtained ($P < 0.001$, 2-tailed test of significance) and indicated a strong correlation between the severity of seizures and the number of small neurons. This finding was consistent with the fact that rats with high ARS displayed the largest number of cells in the IC and those with small ARS had the lowest number of cells.

The size of the IC was similar in all rats based on visual inspection. Our previous study showed that the volume of the IC in the SD was similar to that in the GEPR-9²⁴. This fact is an important point for the present study because our cell counts were from a representative region. If, for example, the size of the IC was smaller in the GEPR-9 as compared to the SD, then our observations would have reflected an increase in cell density rather than an actual increase in cell number. However, since the volume of the IC was similar between the seizing and non-seizing rats, then the increase in cell number was real.

DISCUSSION

The major findings in this study were that: (1)

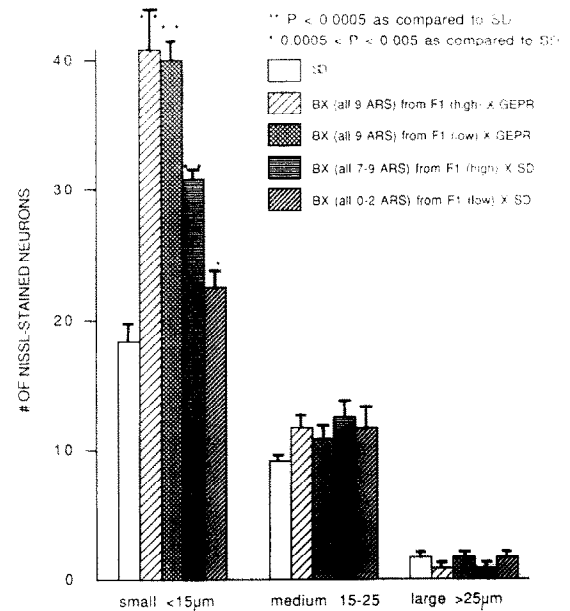


Fig. 7. Histogram showing the mean number of small, medium-sized and large neurons counted in backcrosses (BX) from a representative area in the ventral lateral portion of the central nucleus of the IC. Significant increases in the number of small neurons occur in the F_1 (high) \times GEPR BX, and F_1 (low) \times GEPR (BX) as compared to low-seizing $F_1 \times$ SD BX and SD rat.

the development of seizure behavior in GEPR-9s appears to be dependent on age and previous experience up until 9 weeks of age; (2) the ratio of seizing to non-seizing phenotypes in the F_1 and F_2 generations and in the backcrosses indicates that the gene is dominant; (3) similar numbers of males and females in the F_1 generation produced by reciprocal crosses had similar seizure scores indicating that the seizing trait is autosomal; (4) the F_1 and F_2 generations have different audiogenic seizure profiles than that of either of the progenitor strains; (5) the increase in neuron number previously described in the IC of GEPR-9s is present only in the IC of the seizing offspring but not in the non-seizing progeny; and (6) the number of small cells in the IC is correlated closely with the audiogenic response score (ARS).

The development of audiogenic seizure behavior in the GEPR-9 is similar to that described for the audiogenic seizure susceptible DBA/2J (D2) mouse in that susceptibility is dependent on prior auditory exposure^{8,10,11} and age of testing^{9,26,27}. Our data indicate that the age of onset of seizure susceptibility can be as young as 21 days of age.

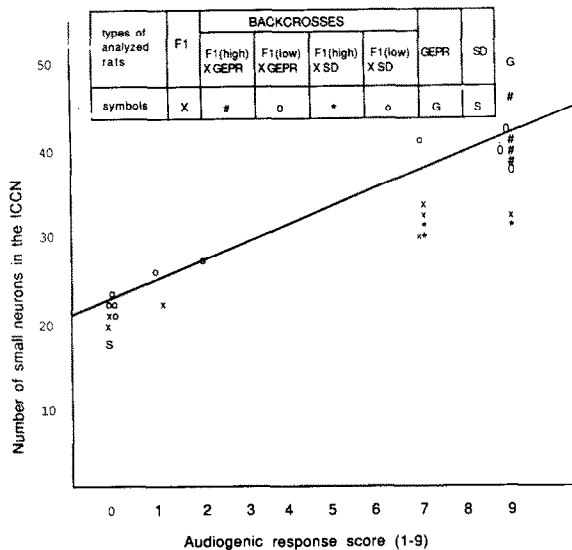


Fig. 8. This graph shows the relationship between the number of small neurons in the IC and the ARS of 25 rats. The coefficient of linear correlation was calculated to be 0.91 using the following equation:

$$r = \frac{S_{xy}}{S_x S_y}$$

where S_{xy} = covariance, and S_x and S_y = S.D. These data indicate a significant correlation between small neuronal number in the IC and ARS.

This is consistent with the data from earlier GEPR-9 colonies (UAZ strain, Genetic registry¹²), with the brief report of Amend et al.¹ and the Krushinsky strain of audiogenic rats¹⁷. DBA/2 mice also exhibit seizures at 21 days of age, but in contrast to the audiogenic seizure rats (both GEPR-9s and the Krushinsky strain), the mice are only maximally susceptible for a short period afterward^{26,29}. However, the seizure behavior in GEPR-9s is not dependent on age alone because not all rats display their seizures by 21 days of age and also, at similar ages after 21 days, a higher percentage of animals that have had previous sound stimulation will seize. Also, seizure behavior is not totally dependent on the number of previous exposures to audiogenic stimuli because only 30% of 26-day-old animals experiencing their fourth test will have an ARS of 9. These data suggest that seizure activity during development may be dependent on developmental events in the CNS as well as environmental events. However, all off-

spring of GEPR-9s display an ARS of 9 at maturity.

The reciprocal crosses between GEPR-9 and SD rats yielded male and female rats with similar distributions of ARS. These data suggest that the trait for seizing is not sex-linked. Therefore, this trait is autosomal.

The fact that the F_1 and F_2 generations have different audiogenic seizure profiles than either of the progenitor strains suggests that the inheritance of AGS in rats is not due to a single gene locus. If a single gene was responsible for the difference in audiogenic susceptibility between the GEPR-9s and SD progenitor strains, there would not be a continuous distribution of seizure scores observed among the F_1 and F_2 progeny. In addition to the new audiogenic seizure phenotypes exhibited by the F_1 and F_2 progeny, a high degree of variability of seizure activity within and between litters was present.

A polygenetic mode of inheritance has been suggested for the audiogenic seizure mouse (DBA/2J) by Seyfried et al.³⁰. Their conjecture was based on an analysis of F_1 and F_2 progeny as well as backcross and recombinant inbred strains. These authors argued that if the inheritance of audiogenic seizures were under the control of one gene, then there would be a 1:1 phenotypic ratio of F_1 -like to DBA/2J-like mice in the $F_1 \times$ DBA/2J backcross, and phenotypes similar to either of the progenitor strains would be observed in the recombinant inbred strains. Similar to our study, the F_1 generation of mice displayed new phenotypes in the recombinant inbred strains. Furthermore the ratio of phenotypes of F_1 -like to DBA/2J-like mice in the $F_1 \times$ DBA/2J mice was not 1:1. This latter finding has also been found in similar studies in the mouse by Schlesinger et al.²⁶ and Collins⁴. Indeed, the ratio of phenotypes in the progeny of the backcrosses in the present study was also not 1:1. All the progeny from the backcross between GEPR-9 and high- and low-seizing F_1 displayed maximal ARS of 9 which was characteristic of the GEPR-9s. Backcross progeny from F_1 high-seizing \times SD rats showed relatively high ARS (7–9) and the last group from the F_1 low-seizing \times SD rat backcross showed relatively low ARS (0–2). These findings suggest that a major gene may underlie audiogenic

seizure susceptibility in the GEPR-9.

Although a similar mode of inheritance for AGS may exist in both mice and rats, the genetic factors influencing seizure behavior are probably different in some respects. This conclusion is based on several reports that indicate differences between the characteristics of AGS mice and rats. As already mentioned, rats are susceptible throughout their lifetime beginning as early as 21 days of age, whereas certain mice are only susceptible from 21 to 28 days of age. Finally, a norepinephrine (NE) deficit is important in seizure susceptibility in the GEPR-9^{13,14} whereas a dopamine deficit is important in mice²⁸.

It is likely that an interplay of transmitter defects influence seizure behavior in the GEPR-9 because in addition to the increase in the number of GABAergic neurons in the IC, NE and serotonin deficits are present in the CNS of GEPR-9s and susceptible progeny^{14,18}. Jobe et al.¹⁴ have suggested that the NE deficit is a determinant of seizure susceptibility as well as severity based on these data and other data that indicate that manipulations that increase NE content in the brain protect against seizures in GEPR-9s. Conversely, depletion of NE prior to sound stimulation increases the severity of seizures in GEPR-9s and elicits seizures in the non-susceptible progeny of susceptible parents. However, it has no effect in control animals exposed to sound stimulation. These observations have led Jobe et al.^{13,14} to suggest that the expression of seizure behavior in rats is dependent on more than one genetic factor.

The increase in cell number in the IC of seizing rats represents another defect in the CNS of these animals^{24,25}. Since an increase in the number of small cells is present in GEPR-9s, seizing offspring²⁵ and the young, naive offspring of seizing parents²⁴ and is highly correlated with seizure severity (present study), it is possible that this defect may play a direct role in the expression of seizure behavior. This increase in small neuronal number is probably reflecting an increase of GABAergic neurons because most GABA neurons in the IC of GEPR-9s and SD rats are small neurons²⁵ and increased levels of GABA are found in the central nucleus of the IC²¹. Therefore, the increase in the number of all neurons in the seizing F₂ and back-cross animals probably reflects an increase in the number of GABAergic neurons. The role of the increased number of neurons and GABAergic neurons in the IC of GEPR-9s and in seizing offspring is unclear. This anatomical defect could be a compensatory mechanism for the other neurotransmitter abnormalities that are present in the CNS of seizing animals^{13,14,18}. Alternatively, the increase in cell number may play a more direct role in seizure induction through mechanisms that have not yet been elucidated.

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